AD NUMBER

ADB240398

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Aug 98. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, dtd 21 Feb 2003

AD_____

Grant Number DAMD17-96-1-6123

TITLE: Design, Synthesis, and Testing of Breast Cancer Angiogenesis Inhibitors

PRINCIPAL INVESTIGATOR: Edward L. Schwartz, Ph.D.

CONTRACTING ORGANIZATION: Montefiore Medical Center
Bronx, New York 10467

REPORT DATE: August 1998

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1998	Annual (15 Jul 97 - 14 Jul 98)

4. TITLE AND SUBTITLE
Design, Synthesis, and Testing of Breast Cancer Angiogenesis Inhibitors

5. FUNDING NUMBERS
DAMD17-96-1-6123

6. AUTHOR(S)
Edward L. Schwartz, Ph.D

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Montefiore Medical Center
Bronx, NY 10467

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, August 1998). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Tumor angiogenesis is the process by which a growing tumor mass recruits the new blood vessels required for its continued growth, and through which the tumor can spread to distant sites. Indeed the neovascularization process is thought to be one of the rate limiting steps for the growth of primary and metastatic breast tumors. This proposal will focus on the development of inhibitors of a particular angiogenic factor, thymidine phosphorylase/platelet-derived endothelial cell growth factor (TP/PD-ECGF). These inhibitors will target the enzymatic activity of TP/PD-ECGF, since it has been found to be required for angiogenesis. In the past year, several compounds were synthesized as potential TP inhibitors, including one having high potency as an enzyme inhibitor *in vitro* (IC_{50} = 1.5 μM). This compound will now be tested in biological assays.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer Angiogenesis Thymidine phosphorylase	11
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

__✓__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Ed Schmitt 8/12/93

PI - Signature Date

Table of Contents

Introduction. There is a need for alternative approaches to treat metastatic breast cancer. One rapidly developing area of investigation is the study of tumor angiogenesis, the process by which a growing tumor mass recruits the new blood vessels required for its continued growth, and through which the tumor can spread to distant sites. Indeed the neovascularization process is thought to be one of the rate limiting steps for the growth of primary and metastatic tumors.[1-2] Most studies have demonstrated the importance of angiogenesis in the progression of human breast malignancies and found the extent of vascularization to be correlated with tumor size and an indicator of node metastasis.[3-6] Quantitative pathology studies in early breast cancer have shown a worse prognosis for those patients with highly vascular tumors, and similar results were obtained in studies of both node-negative and node-positive breast carcinomas.[5-8] Further, the prognostic influence of tumor angiogenesis was independent of other prognostic indicators.

Several polypeptides and growth factors that are produced by breast cancer epithelial and stromal cells have been identified as having endothelial cell mitogenicity and angiogenic activity: TGFα and β (transforming growth factor), VEGF (vascular endothelial growth factor), PD-ECGF (platelet-derived endothelial cell growth factor), FGF (fibroblast growth factor) and TNFα (tumor necrosis factor).[1,9] Our studies are focusing on the angiogenic factor PD-ECGF, based on recent evidence demonstrating its role in experimental and human cancer, and the finding that PD-ECGF is identical to human thymidine phosphorylase (TP), an enzyme that catalyzes the reversible conversion of thymine to thymidine.[10-12] When transfected into NIH 3T3 cells, TP/PD-ECGF was found to increase the vascularization of tumors growing in nude mice after sc inoculation.[13] Similarly, overexpressing TP/PD-ECGF in MCF7 breast carcinoma cells markedly increased tumor growth *in vivo*, although it had no effect on the growth of the cells *in vitro*.[14] Western blot analysis of primary human breast tissue showed that TP/PD-ECGF expression was elevated in the tumors compared to the normal tissue[14], a finding which provides clinical relevance to the transfection experiments.

Studies suggest that the angiogenic and endothelial cell chemotactic activities of PD-ECGF are dependent upon its enzymatic activity, and this has been confirmed with site-directed mutagenesis studies.[14,15-17] Of the angiogenic factors identified to date, TP/PD-ECGF is the only one in which an enzymatic activity of the factor is required for angiogenic activity. These observations serve as the basis for our hypothesis that inhibition of the catalytic activity of TP/PD-ECGF will also block its angiogenic properties. By synthesizing inhibitors of TP/PD-ECGF, we will be able to test this hypothesis and provide a basis for the development of a novel class of antitumor agents.

PROPRIETARY

Progress in the Synthesis and Evaluation of Target Compounds

Our original proposal suggested the synthesis of four structurally distinct classes of pyrimidines or pyrimidine nucleosides to be evaluated as potential TP/PD-ECGF inhibitors (I - IV shown in Fig. 1). We reported in last year's progress report significant headway in the synthesis and evaluation of classes I and III. The early portion of this year's efforts has focused therefore on the synthesis of members of the two remaining classes II and IV.

Fig. 1

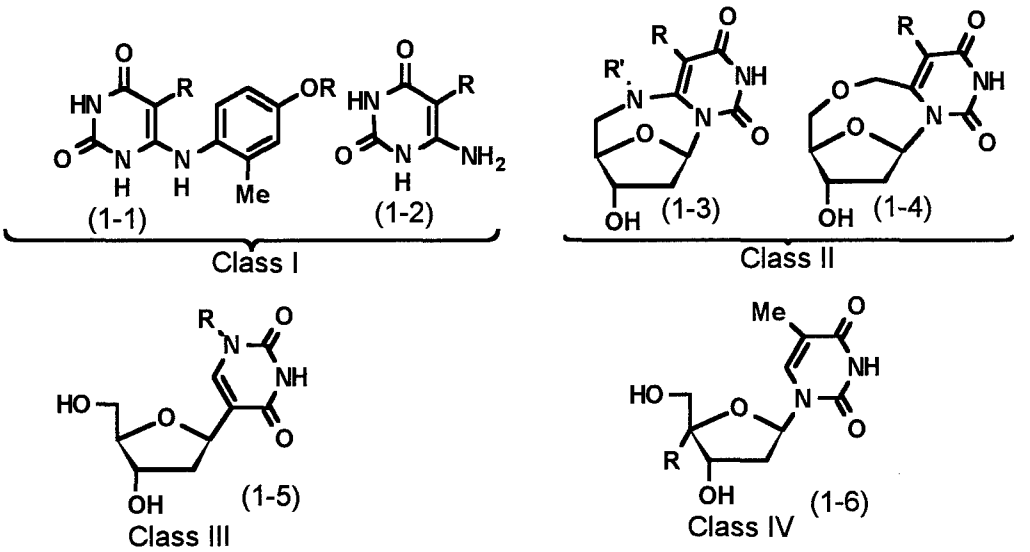
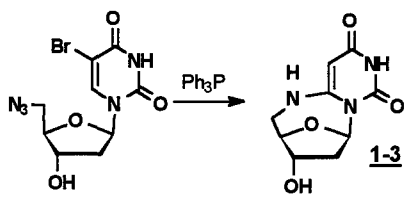


Figure 1: Classes of compounds to be synthesized and tested as inhibitors of TP/PD-ECGF catalytic activity.

In our investigation of new analogs belonging to class II, we have synthesized key intermediates which are, as illustrated below, only one step removed from target analog **1-3** (where R'=R=H in fig. 1). The remaining step involves a final cyclization via reduction of the azido groups to NH_2 with triphenylphosphine to afford the desired product.



Significant progress has also been achieved in the synthesis of derivatives of class IV (see Fig. 2) where a series of synthetic conversions starting with thymidine (**2-1**) have resulted, as originally planned, in the synthesis of key intermediate **2-8**. The latter should be readily convertible to the desired potential bisubstrate inhibitors **2-13** as shown.

Fig. 2

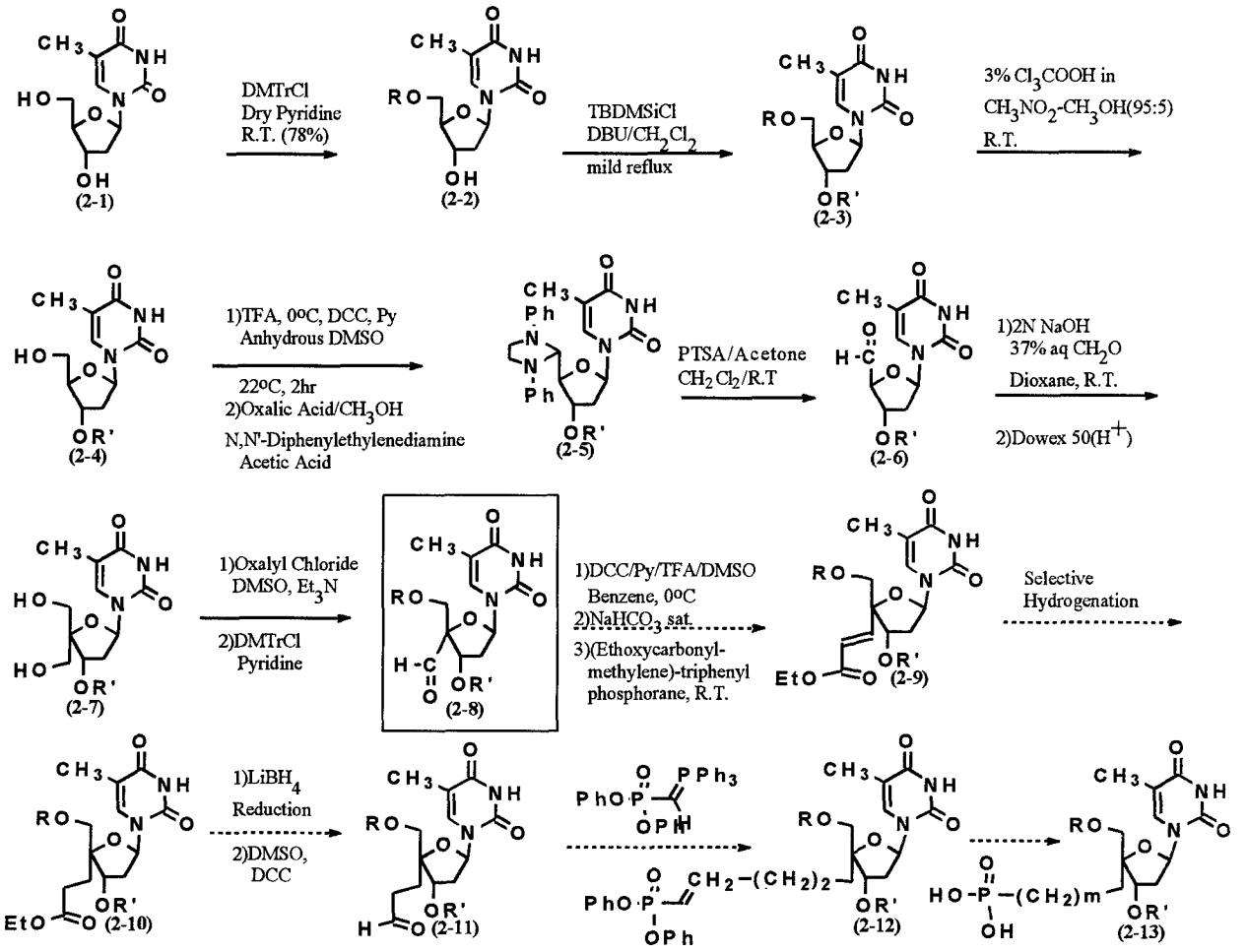
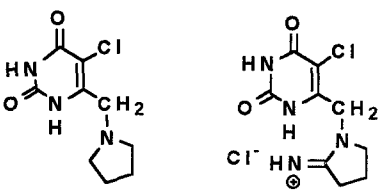


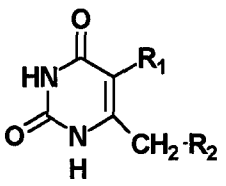
Figure 2: Synthetic route for the synthesis of class IV compounds.



One of the most significant recent developments in the area of TP inhibitors as antiangiogenic agents was the recent report by a Japanese pharmaceutical company (Taiho Pharmaceuticals) of a series of uracil derivatives (shown in figure to right) that had pronounced TP inhibitory activity (18). We have decided to incorporate this important insight into our synthetic chemistry program, and therefore have established a new class of compounds to be evaluated (Class V, see Table 1, below). While somewhat similar to the uracil derivatives of class I we had suggested for investigation, these new analogs differ structurally in one significant respect, the incorporation of a methylene group at C-6 which acts as a bridge between the pyrimidine ring and the amines. We first synthesized three such C-6 methylene-containing amines (**V1**, **V2**, **V3**, Table 1) and determined their activity. All three were

poor inhibitors, with K_i greater than 0.3 mM. Suspecting that this may have been due to the lack of a 5-substitution on the analogs, we next prepared compound **V5** with a 5-Cl to mimic the Taiho compound.

The activity of this compound was 1.5 μM, making it the most potent inhibitor of TP we have synthesized to date, surpassing the activity of our previously synthesized 5-bromo-6-aminouracil (K_i = 13 μM) by an order of magnitude. Removal of the 5-Cl moiety (compound **V4**) reduced the K_i to 120 μM, confirming the importance of this substituent. We also synthesized the HCl salt (**V6**) and found it had equivalent inhibitory activity on TP as did **V5**, thereby providing a readily water soluble analog.



Class V

Compound	R_1	R_2
V1	H	—NH—⟨⟩—O—Bn
V2	H	—NH—⟨⟩(CH$_3$, CH$_3$)
V3	H	—NH—nC_4H_8—⟨⟩
V4	H	—N⟨⟩N
V5	Cl	—N⟨⟩N
V6	Cl	—N⟨⟩NH$^+$Cl$^-$

Table 1: Structure of Class V compounds.

In light of these new developments, we have focused our recent efforts on the synthetic investigation of 5-substituted 6-chloromethyluracils for use as precursors to this new type of TP inhibitors. The results of our work in this area are summarized in Fig. 3. We have selected **3-7** and **3-10** as the two precursors of highest priority and most likely to affords highly active analogs. **3-7** was prepared through the sequence shown starting with the reaction of 2-methyl ethylacetoacetate and thiourea. The corresponding 5-Cl derivative **3-10** was obtained originally by chlorination of the commercially available 6-chloromethyluracil **3-11**. In view of its excessive cost, other synthetic approaches to **3-10** have been investigated. We have now prepared it from the more economical and commercially available 6-methyluracil **3-8**. A somewhat longer but possibly better yielding approach should also be possible as shown (**3-8** → **3-12** → **3-13** → **3-14** → **3-10**) which makes use of the same methodology we employed for the 5-Me series. Initial conversions of these precursors to **3-15** and **3-16** have already been carried out.

PROPRIETARY

Fig. 3

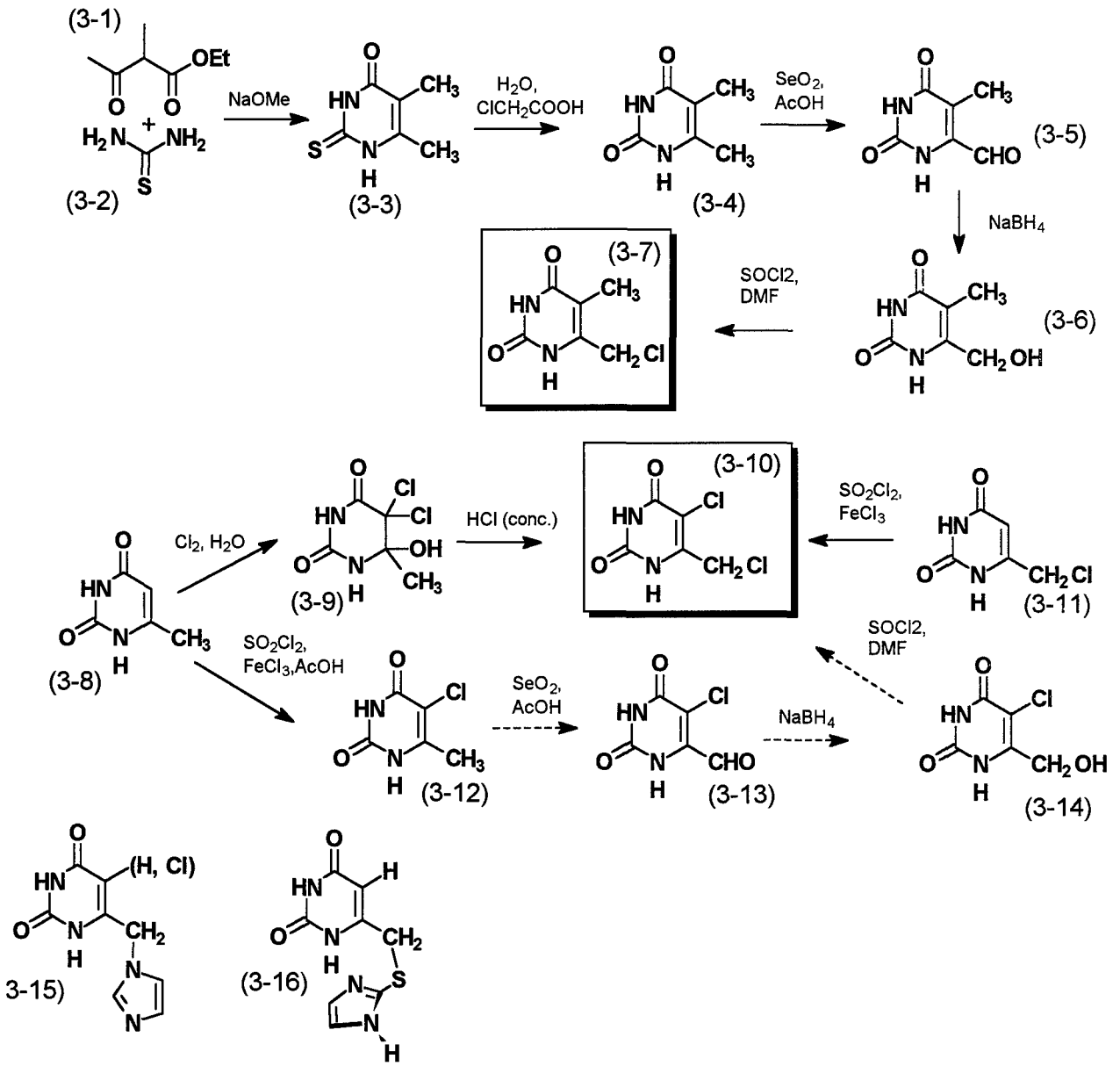


Figure 3: Synthetic routes utilized for obtaining precursors of the new generation of C-6-methylene bridged pyrimidine-to- amino (or amidino or thioimidate) derivatives.

Conclusion. Having now identified and synthesized a highly potent inhibitor of TP (i.e. compound **V5**), we will now begin, in the upcoming year, biological testing as described in the grant proposal.

REFERENCES

1. Folkman J and Shing Y (1992) Angiogenesis. *J. Biol. Chem.* 267:10931-10934.

2. Hayes DF (1994) Angiogeneis and breast cancer. *Hematology-Oncology Clinics of North America* 8:51-71.

3. Weidner N, Semple, JP, Welch WR and Folkman J (1991) Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. *N. Engl. J. Med.* 324:1-8.

4. Horak ER, Leek R, Klenk N, LeJeune S, Smith K, Stuart N, Greenall M, Stepniewska K and Harris AL (1993) Angiogenesis, assessed by platelet/endothelial cell adhesion moleculae antibodies, as indicator of node metastases and survival in breast cancer. *Lancet* 340:1120-1124.

5. Toi M, Kashitani J and Tominaga T (1993) Tumor angiogenesis is an independent prognostic indicator in primary breast carcinoma. *Int. J. Cancer* 55:371-374.

6. Gasparini G, Weidner N, Bevilacqua P. et. al. (1994) Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. *J. Clin. Oncol.* 12:454-466.

7. Fox SB, Leek RD, Smith K, Hollyer J, Greenal M, Harris Al (1994) tumor angiogenesis in node-negative breast carcinomas- relationship with epidermal growth factor receptor, estrogen receptor, and survival. *Breast Cancer Res. Treat.* 29:109-116.

8. Craft PS and Harris AL (1994) Clinical prognostic significance of tumor angiogenesis. *Annals of Oncology* 5:305-311.

9. Hlatky L, Tsionou C, Hahnfeldt P and Coleman CN (1994) Mammary fibroblasts may influence breast tumor angiogeneisis via hypoxia-induced vascular endothelial growth factor up-regulation and protein expression. *Cancer Res.* 54:6083-6086.

10. Moghaddam A and Bicknell R (1992) Expression of PD-ECGF factor in *E. coli* and confirmation of its thymidine phosphorylase activity. *Biochemistry* 31:12141-12146.

11. Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M, Akiyama S-I, Fukui K, Ishizawa M and Yamada Y (1992) Angiogenic factor. *Nature* 356:668.

12. Sumizawa T, Furukawa T,Haraguchi M, Yoshimura A, Takeysu A, Ishizawa M, Yamada Y and Akiyama S-I (1993) Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor. *J. Biochem.* 114: 9-14.

13. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, Usuki K, Takaku F, Risau W and Heldin C-H (1989) Identification of angiogenic activity and the

cloning and expression of platelet-derived endothelial cell growth factor. *Nature* 338: 557-562.

14. Moghaddam A, Zhang H-T, Fan T-P D, Hu D-E, Lees VC, Turley H, Fox SB, Gatter KC, Harris AL and Bicknell R (1995) Thymidine phosphorylase is angiogenic and promotes tumor growth. *Proc. Nat. Acad. Sci*. 92:998-1002.

15. Haraguchi M, Miyadera K, Uemura K, Sumizawa T, Furukawa T, Yamada K, Akiyama S-I, and Yamada Y (1992) Angiogenic activity of enzymes. *Nature* 368:198-199.

16. Finnis C, Dodsworth N, Pollitt CE, Carr G and Sleep D (1993) Thymidine phosphorylase activity of platelet-derived endothelial cell growth factor is responsible for endothelial cell mitogenicity. *Eur. J. Biochem.* 212:201-210.

17. Miyadera K, Sumizawa T, Haraguchi M, Yoshida H, Konstanty W, Yamada Y and Akiyama S (1995) Role of thymidine phosphorylase activity in the angiogenic effect of platelet-derived endothelial cell growth factor/thymidine phosphorylase. *Cancer Res.* 55:1687-1690.

18. Matsushita S, Tari A, Furukawa T, Sumizawa T, Miyadera K, Yamada Y, Kanzaki T and Akiyama S (1998) The mechanism of cytotoxic action of 5-fluoro-2'-deoxyuridine in thymidine phosphorylase expressing cell lines. Proc. Amer. Assoc. Cancer Res. 39:434.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 21 Feb 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB263458
ADB282174
ADB270704
ADB282196
ADB264903
ADB268484
ADB282253
ADB282115
ADB263413
ADB269109
ADB282106
ADB262514
ADB282264
ADB256789
ADB251569
ADB258878
ADB282275
ADB270822
ADB282207
ADB257105
ADB281673
ADB254429
ADB282110
ADB262549
ADB268358
ADB257359
ADB265810
ADB282111
ADB273020
ADB282185
ADB266340
ADB262490
ADB266385
ADB282181
ADB262451
ADB266306
ADB260298
ADB269253
ADB282119
ADB261755
ADB257398
ADB267683
ADB282231
ADB234475
ADB247704
ADB258112
ADB267627
ADB282838
ADB233092
ADB263929
ADB282182
ADB257136
ADB282227
ADB282177
ADB263548
ADB246535
ADB282826
ADB282127
ADB271165
ADB282112
ADB255775
ADB265599
ADB282098
ADB232738
ADB243196
ADB257445
ADB267547
ADB277556
ADB239320
ADB253648
ADB282171
ADB233883
ADB257696
ADB232089
ADB240398
ADB261087
ADB249593
ADB264542
ADB282216
ADB261617
ADB269116